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RECOMBINANT PHAGE PROBES FOR *SALMONELLA TYPHIMURIUM* DETECTION

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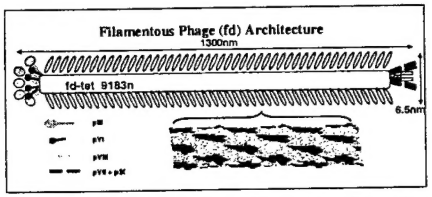
Salmonella typhimurium is a leading cause of inadvertent gastrointestinal foodborne illness in the United States. Although few actual accounts of deliberate food contamination have been documented in the United States, the recent advent of biocrimes and terrorism in our country suggests that this trend will not continue, highlighting the importance of rapidly identifying biological agents, regardless of the contamination origin, as one part of a comprehensive strategic plan to secure the public food supply. There is an urgent need for deployable, real-time threat agent detectors to replace traditional methods of food safety analysis that are slower, labor-intensive, and cost-inefficient. Confirmation of presence in food products can take as long as 48 hours by conventional culture. Current rapid detection initiatives include biosensors that routinely incorporate antibodies as the biorecognition unit. Although sensitive and specific, antibodies are costly and may degrade under unfavorable environmental conditions. We believe that a stable, inexpensive substitute for antibodies is filamentous phage manipulated through phage display technique then affinity selected for specificity to *S. typhimurium* from billion-clone phage landscape libraries. Our results show that recombinant phage affinity selected against *S. typhimurium* can be 12,000-22,000 times for more specific than controls and 10-1000 times more selective for *S. typhimurium* than

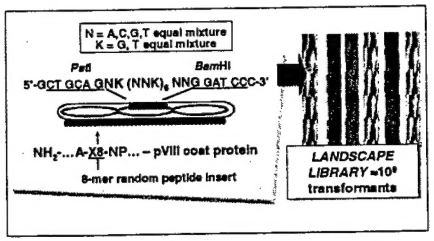
other select enterobacteria. We anticipate that these highly specific, selective phage binders will build upon our current biosensor development initiatives for the rapid detection of biological agents such as *S. typhimurium.*

Introduction

Salmonella typhimurium is a leading cause of foodborne illness in the United States. Confirmation of presence in food products can take 48 hours by conventional culture. Current rapid detection initiatives include biosensors that routinely incorporate antibodies as the biorecognition unit. Although sensitive and specific, antibodies are costly and may degrade under unfavorable environmental conditions. A stable, inexpensive substitute to antibodies is filamentous phage manipulated through phage display technique, then affinity selected for specificity to *S. typhimurium* from billion-clone phage landscape libraries. Results show that recombinant phage can act as highly specific, sensitive probes for direct detection of *S. typhimurium*.



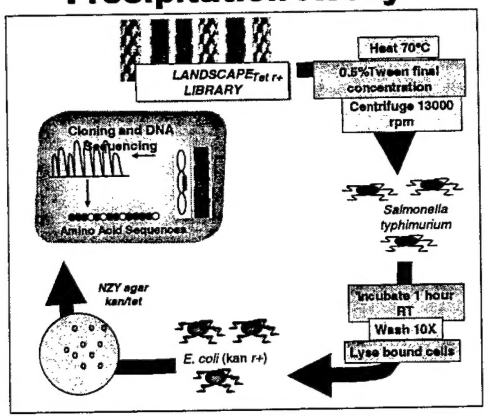
Phage fd-tet outer coat is mainly composed of 4000 50-residue α-helical subunits of **major coat protein pVIII**, which overlap one another to form a tube encasing the viral single-stranded DNA.



In a landscape library, foreign peptides are displayed on every subunit of protein VIII using recombinant technique. The foreign peptides are identical in all 4000 subunits of a single virion, but a landscape library as a whole can represent billions of different peptides altogether.

Methods

Phage Selection Precipitation Assay

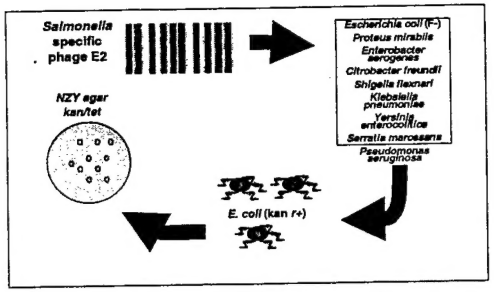


Selection of *S. typhimurium* specific phage clones from the landscape library was performed by affinity selection in solution. Following heat incubation (70° C) and depletion of aggregates by precipitation at high centrifugal speed, a phage library was added to a suspension of *S. typhimurium* for 1 hour to effect cell specific binding. Cells were washed 10X to remove any non-specific binders. Cell bound phage was collected from both eluate and lysate of cells, then amplified in *E. coli*. DNA of recovered phage clones was used to determine the amino acid sequence of their respective PVIII displayed peptides.

Phage specificity

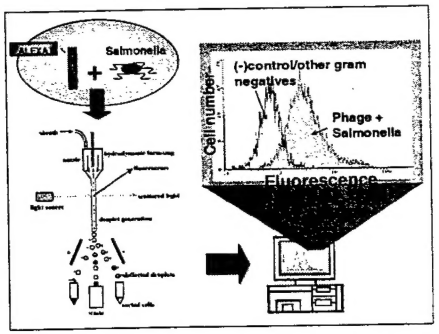
Following recovery of select phage clones, quantitative binding specificity to *S. typhimurium* was determined for each by precipitation assay ("output") (Results, Fig. 1) in comparison with wild-type vector f8-5, which does not express foreign peptides ("input"). Phage titers were determined by infection of *E. coli* K91BK strain and described as ratio of output to input phage (% Yield).

Phage selectivity



Precipitation assay was also utilized to confirm the discriminatory power of the highest specificity phage clone, E2, for *S. typhimurium* in comparison to nine other gram-negative bacteria, predominately *Enterobacteriaceae* selected for their phylogenic relatedness to *S. typhimurium* (Results, Fig. 2). Following incubation of each organism with phage E2, unbound phage were washed from the cells, and the cells were then lysed to recover any membrane bound phage. Phage was then transfected into *E. coli* K91BK for indirect quantitative characterization of direct phage binding to the challenge bacteria.

Binding Confirmation



Since phage clone E2 demonstrated greatest binding (% Yield) to *S. typhimurium*, we utilized this phage clone to visually confirm binding specificity. Fluorescent labeling of E2 using Alexa 488 fluorescent tag conferred an estimated 300 molecules of fluorochrome per phage particle. *S. typhimurium* incubated with the labeled phage was analyzed by fluorescence-activated cell sorting (FACS), TEM and fluorescence microscopy (data not shown).

Results

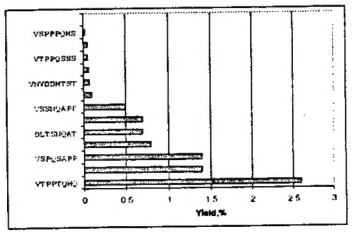


Fig. 1. Specificity of select phage (10^9 CFU/ml) were confirmed for *S. typhimurium* by phage capture, in comparison to ELISA (data not shown), which uses a solid support. Binding of best clones to *S. typhimurium* were 12,000-22,000 times greater than wild-type control phage f8-5, with phage clone E2 (VTPPTQHQ) possessing the highest binding efficiency among all select phage tested.

Conclusions

These results confirm our group's previous research efforts in the development of phage probes for the detection of biological molecules, commending landscape phage as substitute antibodies. We anticipate that these highly specific, selective phage binders will build upon our current biosensor development initiatives for the rapid detection of biological agents such as *S. typhimurium*.

Acknowledgements

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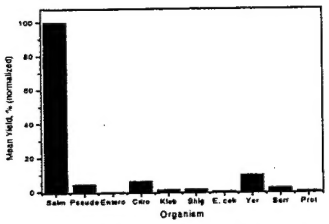


Fig. 2. Precipitation assay demonstrated 90% greater affinity of phage **E2** (mean yield normalized) for *S. typhimurium* in comparison to challenge bacteria. Mean yield % is an average of 3 separate experiments normalized to a maximal mean yield of 2.8% from *S. typhimurium*

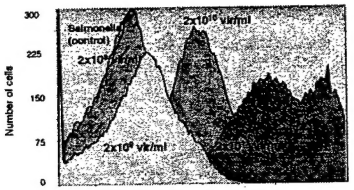
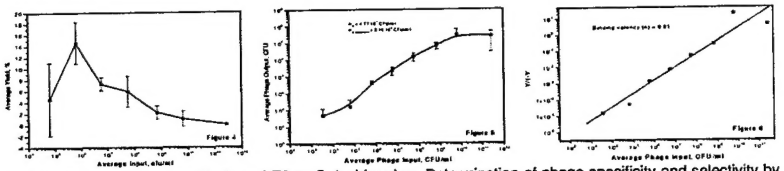


Fig. 3. FACS analysis of phage E2 binding to *S. typhimurium*. Fluorescence associated with cells treated with phage is greater than that of untreated cells and is dependent upon the concentration of phage utilized.



Figs. 4 - 6. Dose-dependent binding of E2 to *S. typhimurium*. Determination of phage specificity and selectivity by precipitation assay, initially mediated at a concentration of 10^9 CFU/ml, resulted in low absolute yield percentage (≈ 2.8% for *S. typhimurium* prior to yield normalization). To determine if dose-dependent concentration was a factor affecting binding, titration of E2 in conjunction with precipitation assay was performed by substituting the single concentration of E2 utilized with concentrations ranging from 10^{12} to 10^1 CFU/ml. A higher yield was demonstrated (Figure 4), with maximum yield at ≈ 10^6 CFU/ml, when ratios of phage to cells approached 1.0, suggesting a "stoichiometric" mode of phage-bacteria interaction. Binding isotherm (Figure 5) confirms concentration-dependent binding of phage to cells in solution. The K_d apparent of the complex calculated from the Hill plot is 3.18 10^9 CFU/ml (= 0.1 nanomolar). Hill coefficient (n = 0.81) estimated from Hill plot (Figure 6) is in good agreement with yield results.